

instructed in the manufacturer protocol (Qiagen Catalog No. 12143). The positive clones then are sequenced using a T7 forward primer (5'-TAATACGACTCACTATAGGG-3') (SEQ ID NO:8) and an M13 reverse primer (5'-CAG[U]TAAACAGCTATGACCAT-3') (SEQ ID NO:9). DNA sequencing
5 identified isolation of a cDNA having the DNA sequence presented in Figure 1 (SEQ ID NO:1) and the amino acid sequence presented in Figure 2 (SEQ ID NO:2).

Example 2-Generation of Mammalian Cells Overexpressing hGAVE3

10 To provide significant quantities of hGAVE3 for further experiments, the cDNA encoding hGAVE3 is cloned into an expression vector and transfected into mammalian cells, such as 293 cells.

To generate mammalian cells overexpressing hGAVE3, mammalian cells are plated in a six-well 35 mm tissue culture plate (3×10^5 mammalian cells per well
15 (ATCC Catalog No. CRL-1573)) in 2 ml of DMEM media (Gibco/BRL, Catalog No. 11765-054) in the presence of 10% fetal bovine serum (Gibco/BRL Catalog No. 1600-044).

The cells then are incubated at 37°C in a CO₂ incubator until the cells are 50-80% confluent. The cloned cDNA nucleic acid sequence of hGAVE3 is inserted
20 using the procedure described above in a pcDNA 3.1 cloning vector (Invitrogen, Catalog No. V790-20). Two µg of the DNA are diluted into 100 µl of serum-free F12 HAM media. Separately, 25 µl of Lipofectamine Reagent (Life Technologies, Catalog No. 18324-020) is diluted into 100 µl of serum-free F12 HAM media. The DNA solution and the Lipofectamine solution then are mixed gently and incubated at room
25 temperature for 45 minutes to allow for the formation of DNA-lipid complexes.

The cells are rinsed once with 2 ml of serum-free F12 HAM media. For each transfection (six transfections in a six-well plate), 0.8 ml of serum-free F12 HAM media are added to the solution containing the DNA-lipid complexes (0.2 ml total volume) and mixed gently. The resulting mixture (hereinafter the “transfection
30 mixture”) then is overlaid (0.8 ml + 0.2 ml) onto the rinsed cells. No anti-bacterial

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